Abstract #1977

ACR-2316: A potentially first-in-class, potent, selective WEE1/PKMYT1 inhibitor rationally designed for superior single agent activity through synergistic disruption of cell cycle checkpoints

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INTRODUCTION

Normal cells, as well as cancer cells, rely on the cell cycle checkpoints (G1/S-, intra-S-, G2/M-checkpoints) to maintain genomic integrity. Cancer cells often have defective G1/S checkpoint and are hypothesized to be particularly sensitive to inhibition of intra-S and G2/M-checkpoints that are governed by WEE1 and PKMYT1.

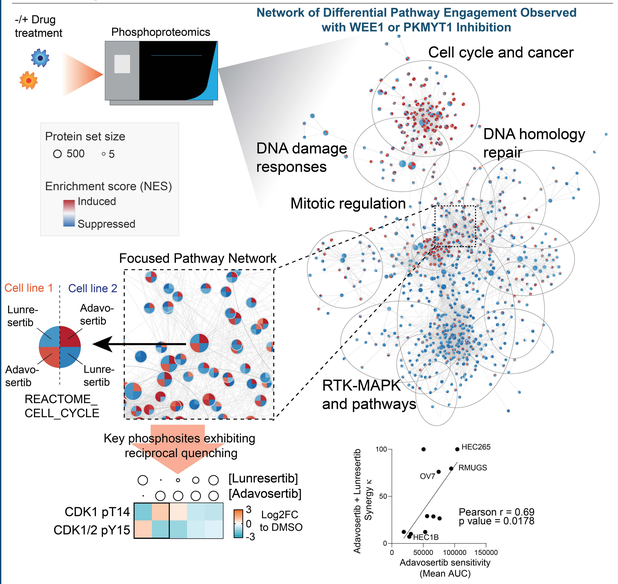
Although some clinical activity with WEE1 inhibitors has been observed in certain tumor types, the challenge to determine upfront which patients will actually benefit from WEE1 inhibition still remains. Furthermore, the majority of patients will at some point develop resistance to targeted therapy. Early understanding of key resistance mechanism enables rational design of either more potent compounds suitable for clinical monotherapy or drug combination therapy to maximize durability of response.

Acrivon's proprietary proteomics-based patient responder identification platform, Acrivon Predictive Precision Proteomics, or AP3, is engineered to measure compound-specific effects on the entire tumor cell protein signaling network and drug-induced resistance mechanisms in an unbiased manner. These distinctive capabilities enable AP3's direct application for biological SAR and drug design optimization for potent single agent activity by ensuring an optimal target selectivity profile to overcome drug-induced resistance mechanisms, with the goal of clinical monotherapy development and potential for the accelerated pathway.

Here we describe the discovery of a selective dual WEE1 and PKMYT1 inhibitor, ACR-2316. This compound was specifically designed using AP3 to overcome limitations of WEE1-specific inhibition through balanced inhibition of PKMYT1, which our AP3 platform uncovered as a dominant WEE1 inhibitor-induced drug resistance. Using structure-guided drug design, we achieved exquisite selectivity for WEE1 and PKMYT1 ensuring primarily mechanism-based reversible adverse events. ACR-2316 demonstrates superior potency and activity compared to current clinical WEE1 or PKMYT1 inhibitors.

RESULTS

Acrivon's Predictive Precision Proteomics Identifies Signaling Pathways Associated with WEE1 or PKMYT1 Inhibitor Resistance

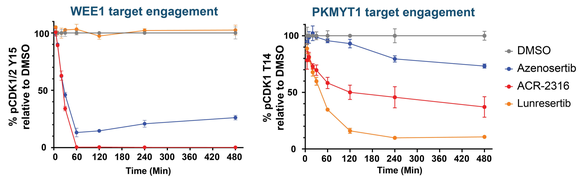


Human tumor cell lines were treated with Adavosertib (200 nM) or Lunresertib (20 nM) for 60 min. Cell lysates were processed for phosphoproteomics mass spectrometry. The drug regulated phosphoproteome was analysed and mapped to cellular pathways to identify resistance mechanisms to WEE1 inhibition. Pathways enriched by WEE1 inhibition included those related to cell cycle, DNA damage response, and mitotic regulation. A subset of these pathways were quenched by PKMYT1 inhibition, with a key phosphosite, CDK1 T14, exhibiting such reciprocal quenching. These data suggest that a combination of WEE1 and PKMYT1 inhibition would be synergistic in terms of fully activating CDK1 and overcoming resistance to WEE1 inhibition. This is supported by correlation between Adavosertib + Lunresertib synergy and WEE1 resistance across human cell line models.

RESULTS

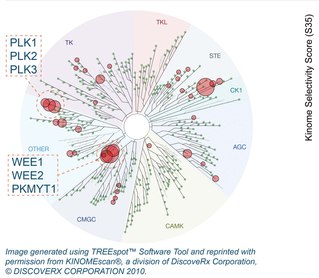
ACR-2316 is a potent dual WEE1/PKMYT1 inhibitor

		ACR-2316	Adavosertib	Azenosertib	Debio-0123	Lunresertib
Biochemical	WEE1 IC_{50}	1 nM	1 nM	2 nM	1 nM	31 nM
	PKMYT1 IC_{50}	27 nM	155 nM	337 nM	>2 µM	10 nM
Cellular target engagement	WEE1 EC_{50} (pCDK1/2 Y15)	2 nM	19 nM	16 nM	109 nM	>5 µM
	PKMYT1 EC_{50} (pCDK1T14)	145 nM	4 µM	>5 µM	>5 µM	11 nM



A LanthaScreen Europium (Eu) Kinase Binding Assay was used to determine binding affinities (IC_{50}) to WEE1 and PKMYT1 for ACR-2316, adavosertib, azenosertib, Debio-0123, and lunresertib. WEE1 target engagement (EC_{50}) was assessed using quantitative high content immunofluorescence imaging of nuclear pCDK1/2 Y15 in a human kidney cancer cell line, and PKMYT1 target engagement (EC_{50}) was assed by the pCDK1 T14 AlphaLISA sandwich immunoassay in a human medulloblastoma cell line (top table). Kinetics of pCDK1/2 Y15 and pCDK1 T14 inhibition in a human kidney cancer cell line treated for 5-480 minutes with 20 nM (left) or 200 nM (right) ACR-2316, azenosertib, or lunresertib (bottom graphs).

ACR-2316 is highly selective for WEE1 and PKMYT1



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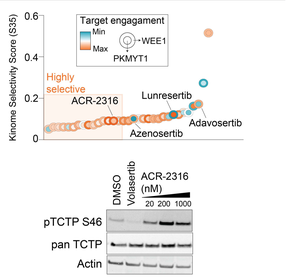
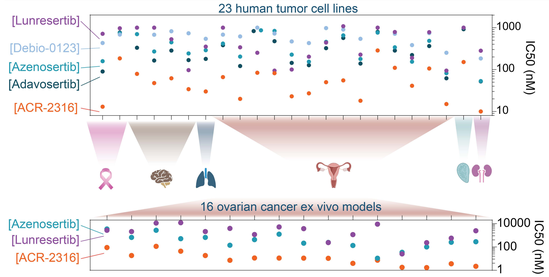


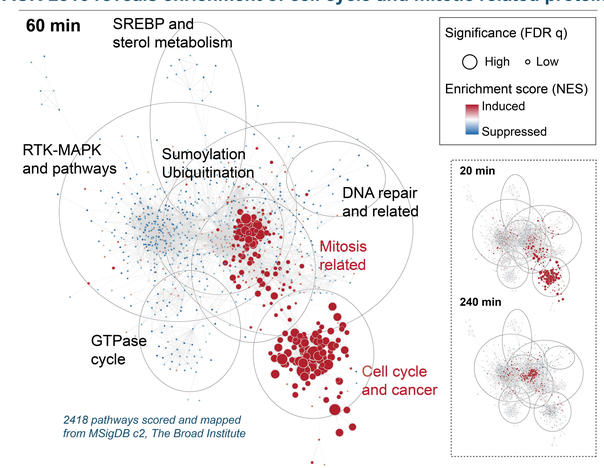
ACR-2316 selectivity was assessed by measuring the binding of ACR-2316 to a comprehensive panel of human kinases included in the scanMAX Kinase Assay Panel at 1 µM drug concentration (left). A compound set of n=33, including ACR-2316 and analogs, azenosertib, adavosertib, and lunresertib were plotted for kinome selectivity score (S35) (y-axis) and target engagement depicted for each compound (upper right). Ovarian cancer cells were treated with PLK1 inhibitor volasertib (1000 nM) and increasing doses of ACR-2316 and blotted for pTCTP S46. ACR-2316 leads to increased pTCTP S46, indicating that ACR-2316 does not inhibit PLK1 activity in cells (lower right).

ACR-2316 is highly potent across human tumor cell lines and patient-derived *ex vivo* tumor models



A wide range of human tumor cell lines, not selected based on underlying genetic mutations, were treated with ACR-2316, azenosertib, adavosertib, Debio-0123, or lunresertib, and viability was assessed after 6 days using Cell Titer Glo (top panel). 16 ovarian cancer patient derived xenograft ex vivo models were treated with ACR-2316, azenosertib, or lunresertib, and viability was assessed after 6 days using Cell Titer Glo (lower panel). ACR-2316 shows superior activity vs benchmarks across all human tumor cell lines and ex vivo models tested.

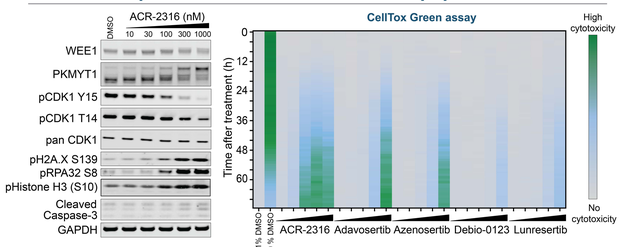
Global phosphoproteome analysis of kidney cancer cells treated with ACR-2316 reveals enrichment of cell cycle and mitotic related proteins

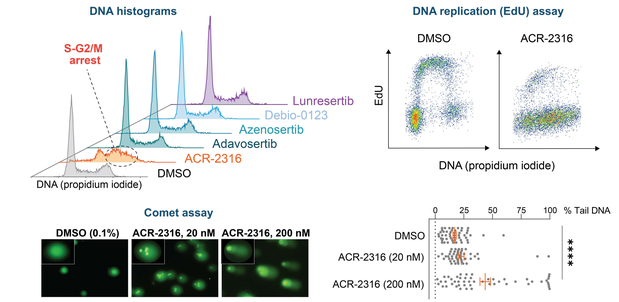


2418 pathways scored and mapped from MSigDB c2, The Broad Institute

ACR-2316 induced phosphorylation pathway activity relating to cell cycle and mitotic pathways. The phosphoproteomes of ACR-2316 vs. DMSO treated human kidney cancer cells were analyzed via dose dependency (20-200-1000 nM), pathway enrichment, and network construction. Pathway activity was assessed after 20 min, 1 hour, and 4 hours, respectively.

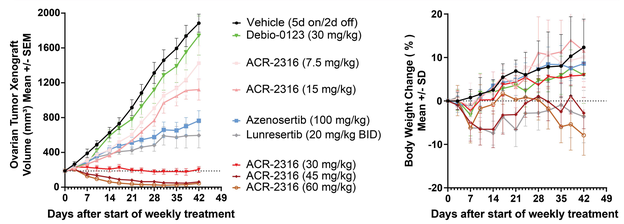
ACR-2316 induces DNA replication stress, DNA damage, premature mitosis and cellular apoptosis





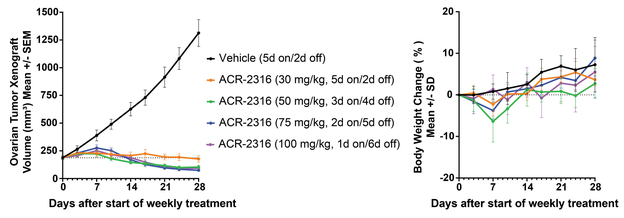
An ovarian cancer cell line was treated with increasing doses of ACR-2316 for 4h and blotted for drug targets (WEE1, PKMYT1), target engagement (pCDK1 Y15/T14), DNA damage (γH2A.X, pRPA32 S8), mitosis (pHistone H3 S10), and apoptosis (cleaved caspase 3) (western blot, upper left). Drug cytotoxicity was measured in ovarian cancer cells using CellTox Green assay for up to 72h (upper right). Disruption of cell cycle was analysed in ovarian cancer cells treated with 100 nM of ACR-2316, adavosertib, azenosertib, Debio-0123, or lunresertib for 24h (DNA histograms, middle panel, left). Disruption of active DNA replication was measured using EdU assay in ovarian cancer cells treated with 100 nM ACR-2316 for 24h (middle panel, right). Induction of DNA breaks was measured using comet assay in ovarian cancer cells treated with 20 nM or 200 nM ACR-2316 for 24h (lower panel left), and the % tail DNA was quantified (lower panel right).

ACR-2316 demonstrates dose-dependent efficacy and near complete regressions in a human ovarian tumor xenograft model



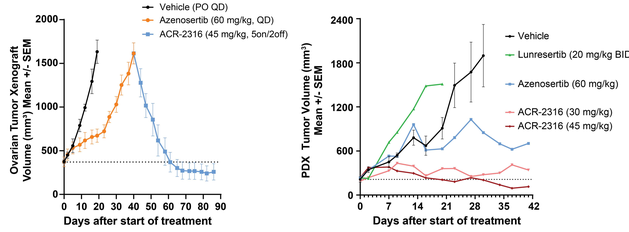
Dose response anti-tumor activity of ACR-2316 versus clinical WEE1 (azenosertib, Debio-0123) and PKMYT1 (lunresertib) inhibitors was assessed in the ovarian tumor xenograft model. All compounds were dosed orally on a 5d on/2d off schedule. ACR-2316 shows superior activity to clinical inhibitors and is well tolerated. Tumor volume data (left) represent Mean ± SEM. Body weight change data (right) represent Mean ± SD.

ACR-2316 demonstrates comparable efficacy with infrequent and frequent dosing schedules



Anti-tumor activity of ACR-2316 with different dosing schedules was tested in the ovarian tumor xenograft model. ACR-2316 was administered orally at 100 mg/kg (1d on/6d off), 75 mg/kg (2d on/5d off), 50 mg/kg (3d on/4d off) or 30 mg/kg (5d on/2d off) and demonstrated comparable efficacy at all doses tested. Tumor volume data (left) represent mean ± SEM. Body weight change data (right) represent Mean ± SD.

ACR-2316 promotes rapid regression of ovarian tumor xenografts progressing on azenosertib, and superior anti-tumor activity in an ovarian PDX model compared to clinical WEE1 or PKMYT1 inhibitors



Mice bearing ovarian tumor xenograft tumors were treated with azenosertib at 60 mg/kg daily until progression to TV~1,500 mm³, then switched to ACR-2316 at 45 mg/kg (5d on/2d off). ACR-2316 demonstrates rapid and deep regression of tumors progressing on azenosertib. Tumor volume data (left) represent mean ± SEM. Mice bearing ovarian cancer patient derived xenograft (PDX) tumors were treated on a 5d on/2d off dosing schedule with ACR-2316 at 30 or 45 mg/kg, azenosertib at 60 mg/kg or lunresertib at 20 mg/kg BID. ACR-2316 shows superior activity to clinical WEE1 and PKMYT1 inhibitors. Tumor volume data (right) represent mean ± SEM.

CONCLUSIONS

- ACR-2316 is a novel, selective dual WEE1/PKMYT1 inhibitor, uniquely designed using AP3 for superior single agent activity to overcome limitations of current WEE1 and PKMYT1 inhibitors, aiming for clinical monotherapy development.
- ACR-2316 demonstrates potent single agent anti-cancer activity and pro-apoptotic cell death in a broad spectrum of in vitro cancer line models including those insensitive to WEE1 or PKMYT1 inhibitors.
- ACR-2316 induces tumor regression in CDX and PDX tumor mouse models with superior activity compared to clinical benchmark inhibitors in head-to-head studies.
- IND enabling toxicity studies demonstrate mechanism based, transient adverse events and ACR-2316 preclinical development is on track for IND submission by Q4 2024.